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Water Regime Affecting the Soil and Plant Nitrogen Availability

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1. Introduction

Nitrogen is a necessary ingredient in soil for agriculturalists to produce high-yielding crops. Europe is one of the world's largest and most productive suppliers of food and fiber (Olesen & Bindi, 2002). These authors provide information that in 2004 Europe produced 21% of global meat production and 20% of global cereal production. About 80% of these global productions have occurred in Europe, defined here as the 25 European countries, EU25 (IPCC, 2007). The productivity of European agriculture is generally high, in particular in Western Europe: average cereal yields in the EU are more than 60% higher than the global average (EFMA, 2010). Some plants (legumes in appropriate conditions) produce their own nitrogen (Dorn, 2011) and some nitrogen is contributed to the soil by rainfall, but these natural sources of nitrogen do not occur in high enough levels for prolific crop production. Many agriculturalists add nitrogen to the soil without regarding the plant needs or nutrient soil status. Addition of nitrogen to the soil helps in the rapid and healthy growth of the plants and thus improves the yields of the crops. It also increases the protein content in the crops as well and food value of crop. However, when N inputs to the soil system exceed crop needs, there is a possibility that excessive amounts of nitrate (NO_3^-) may enter either ground or surface water (O'Leary et al., 2002). Managing N inputs to achieve a balance between profitable crop production and environmentally tolerable levels of NO_3^- in water supplies should be every grower's goal. A recent estimate of the current human population supported by synthetic fertilizer is 48%, 100 years after the invention of the synthesis of ammonia from its elements (Erisman et al., 2008). To maximize crop production, the availability of cheap fertilizer in the industrialized world led to excessive use of nitrogen, resulting in a large nitrogen surplus and increased nitrogen losses. The behavior of N in the soil system is complex, yet an understanding of basic processes (mineralization) is essential for a more efficient N management program. Nitrogen, present or added to the soil is subject to several changes (transformations) that dictate the availability of N to plants and influence the potential movement of NO_3^- to water supplies. Nitrogen can be lost from the soil system in several ways: leaching, denitrification, volatilization, crop removal, soil erosion and runoff. And these ways of N losses from agriculture or industry through the global environment system can cause a numbers of different environmental effects: loss of biodiversity, eutrophication of waters and soils, drinking water pollution, acidify cation,

greenhouse gas emissions, human health risks through exposure to oxidized nitrogen (NO_x), ozone (O_3) and particulates, and destruction of the ozone layer. Nitrogen fertilizer data throughout the world shows that the annual use rate is increasing (Davidson 2009; FAO, 2010). There is no way to totally prevent the movement of some nitrogen forms to water supplies, but sound management practices can keep losses within acceptable limits. Most of the country was developed Nitrogen Fertilizer Management Plan with the purpose of managing N inputs for crop production to prevent degradation of water resources while maintaining farm profitability. The central tool for achievement of this goal is the adoption of Best Management Practices for Nitrogen. Best management practices for N are broadly defined as economically sound, voluntary practices that are capable of minimizing nutrient contamination of surface and groundwater. The primary focus of the BMP's is commercial N fertilizers; however, consideration of other N sources and their associated agronomic practices is necessary for effective total N management. One of these practices will be presented in further text as usage of diagnostic tool for detecting a nutrient status in plant. We will present recent work on different varieties of potato crop fertilized with increasing nitrogen rate. Rapid methods Cardy ion meter and Chlorophyll meter were tested in open field To collect readings as reaction on nutrient regarding the environmental conditions of cultivars growth and in comparison with standard methods. An effective plant nutrient management practice optimizes nitrogen (N) use efficiency for minimized environmental impact, while ensuring an optimum N status of the crop for good product quality and maximum growth. Soil or plant analysis can be used to evaluate the practice; however the use of plant analysis for this purpose has been limited. One reason is lack of reliable reference values for the critical concentration needed for optimal growth and the other is susceptibility of tools on environmental conditions. Nutrients used for plant growth and biomass productions generally come from the internal cycling of reserve materials which require water for their solubility and translocation, so it can be very variable.

2. Crop yield and quality affected by fertilizer use

Crop response to applied N and use efficiency are important criteria for evaluating crop N requirements for maximum economic yield. Recovery of N in crop plants is usually less than 50% worldwide (Fageria & Baligar, 2005). Low recovery of N in annual crop is associated with its loss by volatilization, leaching, surface runoff, denitrification and plant canopy. Low recovery of N is not only responsible for higher cost of crop production, but also for environmental pollution. Hence, improving N use efficiency (NUE) is desirable to improve crop yields and quality, reducing cost of production and maintaining environmental quality. To improve N efficiency in agriculture, integrated N management strategies that take into consideration improved fertilizer along with soil and crop management practices are necessary. Synchrony of N supply with crop demand is essential in order to ensure adequate quantity of uptake and utilization for optimum yield. Practice of reducing NO_3 loss through soil-plant system include improved timing of N application at appropriate rates, using soil tests and plant monitoring, diversifying crop rotations, using cover crops, reducing tillage, optimizing N application techniques, and using nitrification inhibitors. Today many surveys are focused on understanding methods to minimize NO_3 contamination of water resources and professionals put lot of effort to educate the public

about the complexity of the problem and the need for multiple N management practice to solve the problem across agricultural landscapes. The results in the text showed application of tools to monitor the N status of the aboveground canopy of potato, such as chlorophyll readings, sap $\text{NO}_3\text{-N}$ concentrations, N indices to understand plant's demands and status of nutrient as small step to enhance environmental quality and improvement of product quality for the benefit of producers, processors and consumers. For some crops there has to be a balance with maximum yields and quality. Although N deficiencies will decrease yield, excessive N applications can affect the quality of grains, tubers, fruits and other cropping systems. At higher than needed N levels, quality of malting barley (*Hordeum vulgare* L.) can have undesirable high levels of proteins (Zubirinski et al., 1970; Bishop & MacEachern, 1971). Excessive applications of N fertilizer can decrease tuber quality of potatoes (*Solanum tuberosum* L.) (Laughlin, 1971; Painter et al., 1977; Westermann & Kleinkopf, 1985; Errebhi et al., 1998) and sugar beets (Hills & Ulrich, 1971; Cole et al., 1976; Carter & Traveller, 1981; Hill, 1984). Fruit quality can also be affected by high N rates (Locascio et al., 1984). When quality is an important factor in economic returns such as maximizing production, best management practices that can supply and maintain appreciable N levels for maximum yields and quality are needed. These include practices that can provide high N supply during periods of maximum demand and not to supply excessive N that may decrease product quality. Plant analysis has been considered a very promising tool to assess nutritional requirements of plants. Plant analysis, in conjunction with soil testing, becomes a highly useful tool not only in diagnosing the nutritional status but also an aid in management decisions for improving the crop nutrition (Rashid, 2005). Whatever tool is used, the aim is to serve as an indicator of the actual nutrient status of the soil-plant system. Indicators can be used to evaluate the actual plant nutrient management practice (diagnostic indicators) or to give predictive information such as information on the actual fertilizer requirement for the next application (prognostic indicators) (Lewis, 1993; Schröder et al., 2000). The use of indicators to evaluate the actual practice implies a participatory learning process by which the farmer's motivation for a change is encouraged (Röling & Wagemakers, 2000). The management of plant nutrients can be successively improved by evaluation of the fertilizer practice. Generally, an ideal indicator must be reproducible (Schröder et al., 2000). For evaluation of the nutrient status, the indicator should interpret the actual nutrient status of the soil-plant system in the same manner over different sites and years. Plant analysis are widely used for identifying plant nutrition deficiencies and disturbances in crops but only to a lesser degree for routine evaluation of the plant nutrient status for adequate plant nutrient management. An evaluation of the nutrient status is made possible only by relating the actual status to a standard (Ekblad, 2007). The material of living plants were consisted of organic matter, water and minerals. The relative amounts of these three components may vary, but for green plant material, water is always present in the highest proportion and the minerals in the lowest. The percentage distribution of these three components is in the following order of magnitude: water 700 mg g⁻¹ fresh matter, organic material 270 mg g⁻¹ fresh matter and minerals 30 mg g⁻¹ fresh matter. The minerals makes only a comparatively small proportion of the dry matter. They are nevertheless of extreme importance because they enable the plant to build up organic material (photosynthesis). But the ratios of these three components of plant material are highly dependent on environmental conditions.

3. Nitrogen affecting the water quality

The enrichment of nitrogen in the aquatic system impairs the water quality of rivers, lakes, aquifers and coastal and marine waters contributing to the phenomenon of eutrophication (European Environment Agency, 2001). Groundwater is an important resource in Europe, providing water for domestic use for about two third of the population but groundwater is a finite and slowly renewed resource and over exploitation associated with a degradation of water quality is putting in danger an important source of drinking water. In Europe, groundwater nitrate concentrations have remained stable and high in some regions (European Environment Agency, 2005). Most of the nitrates found in groundwater are thus of anthropogenic origin and mostly related to agricultural activities. Indeed nitrogen surplus in agricultural land can be removed by surface runoff, leaching to the aquifer, and loss to the atmosphere or can be stored in the soil–water system. Nitrogen surplus from agriculture are still high in many countries and huge quantities of nitrogen are stored in the soil or aquifers (Grizzetti et al., 2008). There are some major concerns as the Eastern countries will probably intensify their agriculture and thus their fertilization rate in the near future and some countries of Western Europe have not seen their nitrogen surplus decrease but rather stabilize at high levels. Efforts have been taken through conventions or the application of binding Directives and still Europe's waters are suffering from excess nitrogen. Currently in many countries there are strict limits on the permissible concentration of nitrate in drinking water and in many surface waters. The limit is 50 mg NO_3/l in the European Drinking Water Directive (Directive 98/83/EC) and 44 mg NO_3/l in the United States (equivalent to 11.3 mg N/l and 10 mg N/l, respectively). These limits are in agreement with WHO recommendations established in 1970 and recently reviewed and reconfirmed (WHO, 2007; the exact formulation of the standard is that the sum of $\text{NO}_3/50 + \text{NO}_2/3$ should not exceed 1). The European Nitrates Directive also sets a limit concentration of 50 mg NO_3/l for groundwater and surface water as a threshold value for Member States to protect water bodies. Today the agriculture is identified as the single largest source of impairments for water sources. Nitrogen is one of the most abundant elements. About 80 percent of the air we breathe is nitrogen. It is found in the cells of all living things and is a major component of proteins. Inorganic nitrogen may exist in the free form as a gas N_2 or as nitrate NO_3^- , nitrite NO_2^- or ammonia NH_3^+ . The rate, time and method of nitrogen application can affect the risk of nitrogen loss to surface water and groundwater. Leaching of nitrate to groundwater and nitrate in subsurface drainage is typically more concentrated with higher nitrogen rates, but the effect of nitrogen rate varies across locations. Soil nitrogen levels and crop needs often are not defined by field borders. Variable types N fertilizers allow farmers to apply fertilizer when and as needed, thus reducing nitrogen loss of water resources, reducing loss of nitrogen to water resources. Achieving a balance of productivity, profit and water quality protection is the goal for nitrogen rate optimization. Not only does the nitrogen impact the water quality, But also have effect on human health. There are two main health issues related to nitrate in drinking water: the linkage with infant methaemoglobinaemia, also known as blue baby syndrome and with cancers, for example of the digestive tract (Ward et al., 2005).

4. Plant analysis as a diagnostic tool for plants nutrition disorder

Plant tissue analysis shows the nutrient status of plants at the time of sampling. This, in turn, shows whether soil nutrient supplies are adequate. In addition, plant tissue analysis will detect

unseen deficiencies and may confirm visual symptoms of deficiencies. Toxic levels also may be detected. Though usually used as a diagnostic tool for future correction of nutrient problems, plant tissue analysis from young plants will allow a corrective fertilizer application that same season. Using established critical or standard values, or sufficiency range, a comparison is made between the laboratory analysis results with one or more of these known values or ranges in order to assess the plant's nutritional status (Jones et al., 1991; Kelling et al., 2000; Rashid, 2005). The use of plant analysis as a diagnostic tool has a history dating back to studies of plant ash content in the early 1800's. While working on the composition of plant ash, researchers recognized the existing relationships between yield and the nutrient concentrations in plant tissues. Quantitative methods for interpreting these relationships in a manner that could be used for assessing plant nutrient status arose from the work of Macy (1936). Since then, much effort has been directed towards plant analysis as diagnostic tool. Plant analysis is carried out as a series of steps that include sampling and sample preparation followed by laboratory analysis and interpretation of analytical data. Each step is equally important to the success of the technique employed for diagnosing nutritional disorders. Since plant species, plant age, plant part, sampling time and applied fertilizer are all variables that affect the interpretation of the analytical data; careful sampling is highly important (Jones et al., 1991). Surveys of nutrient concentrations in "deficient" and "adequate" N rate for potato crop have been used to establish standard nutrient concentrations. Vegetation period should also be taken in consideration. Nutrient deficiencies are often difficult to identify because a number of interacting factors may cause similar symptoms. Factors such as pests, unfavorable placement, soil chemical properties, soil compaction, or moisture stress can prevent nutrient uptake even if nutrients are plentiful in the soil. Plant tissue analysis will indicate if the crop took up soil-applied nutrients. For perennial fruit crops (blueberries, strawberries, apples, grapes, peach, etc.), these analysis are the best way to monitor the plant's nutrient needs. Plant analysis can be used to fine tune the efficiency of a fertilizer program before nutrient deficiency symptoms occur and is very useful in improving the fruit quality and yield. From emergence through the first few weeks of growth depending on phenophases of sampling crop, plant analysis is helpful in identifying nutrient uptake. Testing the leaf samples at this early stage may indicate where additional nitrogen should be applied. This is a way to determine the cost-effectiveness of the additional application of fertilizer. In field research on different location we have tested two diagnostic tools in interpretation nitrogen status in three varieties of potato crop. Different location were used to compare variation in values depending on micro-climates condition of growth even the same fertilization treatments were applied. Varieties with different vegetation period were also compared to evaluate the distinct in nutrient accumulation rate. For measurement of nutrient status it have been used Chlorophyll meter for evaluation of chlorophyll index in potato leaf and Cardy ion meter for evaluation of nitrate-nitrogen concentration in petiole sap of plant. Data from both measurements were compared to the laboratory analysed leaf on total nitrogen concentration.

4.1 Rapid nitrogen diagnostic tools

Potato plants deficient in N have pale green leaves, poor growth and reduced yield. Excess N fertilizer application increases the chances of surface and ground water contamination. Many farmers in developed countries use a pre-season soil nitrate test to adjust N fertilizer rates to specific potato yield goals. Use of new instruments that instantly estimate potato plant N levels may allow farmers to precisely target N fertilizer applications to changing weather and

crop conditions during the growing season. Fertilizer N utilization efficiency increased when N is applied near the time of greatest need of the crop. Beside basic fertilization application for potato it is common additional treatment usually 45 day after the planting. We have used in our survey two meters that estimate plant N Chlorophyll Meter –(Soil Plant Analysis Development-SPAD 502 meter, Minolta, Osaka,. Japan) (Figure 1) and Cardy-ion Meter (Figure 2). Values obtained by these meters were compared to the standard laboratory measurement of total leaf N expressed on dry weight basis (%) by Kjeldahl method (AOAC, 1970).

The Chlorophyll Meter sensor clamps on intact leaves and instantly measures leaf chlorophyll "greenness". Because there is a close relationship between chlorophyll level and leaf N the Chlorophyll Meter readings (SPAD values) are an indicator of leaf N level (Spectrum technologies, 2011). It is important to take the reading on about the same location on each leaf. It works well to collect the reading from a point one-half the distance from the leaf tip to the collar and halfway between the leaf margin or edge and the leaf midrib. Chlorophyll meter readings are usually stable during the day unless plants are under water stress. As long as readings are collected from the reference strip and the adjacent bulk field at about the same time, the comparison is valid. It is best to avoid collecting readings when moisture is on the leaves (i.e., after a rain or sprinkler irrigation or in the early morning) or when plants are under drought stress as this can distort the readings. Meters should not be subjected to extreme temperature changes before making measurements. Although the chlorophyll meter enables user to quickly and easily measure leaf greenness which is affected by leaf chlorophyll content, several other factor affect SPAD values. Differences in leaf thickness reflected in specific leaf weight are largely responsible for variations in the relationship between N content and SPAD values (Peng et al., 1993). Moreover, the linear relationship of SPAD values and N status in crops varies, depending on growth stages and cultivars. Finally, environmental and stress factor caused by excess or limited water, deficiency of nutrient other then N and pest and diseases can also confound the SPAD readings (Smeal and Zhang, 1994). Producers should recognize this as another tool that may complement, but does not replace, other aspects of sound N management. One soil scientist said it succinctly: "Use the chlorophyll meter to schedule your last 50 lbs N/acre, not your first." Because it is suggested that at least one-half to three-quarters of the total fertilizer N should be applied to the entire field prior to the stage of three leaf to ensure the chlorophyll meter technique effective.

The Cardy ion meter has a sensor that measures the nitrate concentration in liquid extracts of plant tissues. Nitrate moves from roots to leaves in potato plant where were assimilated into amino acids and proteins. While plants take up nitrogen in both the ammonium and nitrate forms, nitrate is usually more abundant than ammonium so nitrogen tests measure nitrate rather than ammonium. Under conventional fertilizer practices, plant tissue contains high levels of nitrate which is a good indicator of the nutrient status of the plant. Soils also contain varying amounts of ammonium forms of nitrogen, which bacteria convert to nitrate forms over time. But Cardy ion meter do not measure ammonium nitrogen and therefore some underestimate of the nitrogen may become available to the plants during the growing season. The Cardy ion meter will measure the leaf nitrate-N concentration (in ppm or mg kg⁻¹ fresh weight) but does not measure leaf amino acid and protein level. A portable Cardy ion meter with selective electrode has recently been developed that can directly measure

NO_3^- and NO_3^{--}N form of nitrogen present in fresh samples of squeezed petiole sap. This Cardy ion meter offers immediate results of in-season crop N status. Therefore, adjustments in N fertilization can be made before the crop experiences N deficiencies or excessive N applications which may lead to enhanced vegetative growth, yield reductions, and/or delayed maturity.



Fig. 1. Measurements of Chlorophyll index on potato leafs by Chlorophyll meter.



Fig. 2. Measurements of $\text{NO}_3\text{-N}$ from petiole sap of potato crop by Cardy ion meter.

5. Materials and methods

The trial fields with potato variety planted on three different location fertilized with increasing N rate were conducted in Bosnia and Herzegovina in 2007. Data were obtained using the rapid diagnostic tools for plant material analysis in comparison to the standard measurement.

5.1 Experimental stations

Trial field were performed on three different locations Mostar (L1), Malo Polje (L2) and Stolac (L3) away from each other around 20 km. Each experimental station was set up on 300 m² of surface. All three locations are in Herzegovina region and survey was conducted in 2007. In trial field we have used common potato varieties Adora, Liseta and Romano. Potato seed were machinery sown with in row seed space 0.18-0.20m and between rows seed space 0.65m. Three fertilization treatments (0, 100, 200 kg N ha⁻¹) were used in a split-plot design with three replications. N fertilization treatments were estimated according to the soil analyses, adding the one half of the total N amount before planting and other 45 days after emergency of crop. For basic fertilization we have used NPK formulation 7:20:30 with additional dressing UREE and KAN. Potato plants grown under various N fertilizers were sampled at the 4th growth stages (65, 75, 85 and 95 days after sowing-DAS).

5.2 Collection of the samples

Chlorophyll and Cardy ion meters are used in field trial with potato crop tasting the effectives of tools in evaluation of fertilization rate. The youngest fully-expanded leaf (3-4 from the top of the canopy) was used for obtaining the values measured by Chlorophyll Meter (30 readings are collected for average values per sample). Chlorophyll measurements were preformed during the morning period when the temperatures are not high starting with sampling from the period of appearance the first flowers (65 DAS). Each SPAD value was the mean of the measurement on 6 leaflets. After this samples were stored in paper bag to the small hand fridge and later in laboratory we have proceed measurement of the NO₃-N values from the petioles sap of the same samples. From all leaflets petioles were removed by cutting and squeezing the sap by hydraulic plant press. The petiole sap was used for obtaining the values of NO₃-N concentrations by the Cardy ion meter. Both methods are compared to the standard laboratory N (percentage of total leaf N expressed on dry weight basis) measurement expecting a reliable data on N plant status as these methods are not yet tested for each crop and values can varied depending on environmental conditions. Providing the reliable data by at least one of these meters we can replace the long lasting and expensive laboratory plant sample measurement. Nitrogen concentration in plants is normally determined by expensive and time consuming chemical analyses (AOAC, 1970). As an alternative, Chlorophyll and Cardy ion meter readings in leaf and petiole sap were proposed, but these assays are not always satisfactory.

5.3 Plant material

For plant material we have used common potato varieties on Herzegovina market: cultivar Adora, Liseta and Romano. Cultivar Adora has very short vegetation about 80 days. Tubers are oval, light yellow skin colored and smooth skin with medium shallow eyes. Plants are erected medium high with smaller number of thinly shoots. Leafs are larger, dark green, half-open. This cultivar could have a high yield smaller tuber number per plant. Dry matter of tuber is lower. Cultivar Liseta has early vegetation from 95 to 100 days. Tubers are elongated-oval, smooth to medium smooth skin, light yellow skin colored and with shallow eyes. Plants are half-erected, medium-high with light colored shoots. Leafs are light green medium-open. This cultivar could have a high yield with higher tuber number per plant. Dry matter of tuber is higher. Cultivar Romano has same vegetation period as cultivar Liseta (95-100 days). Tubers are around and oval, smooth light red colored skin with relative

depth eyes. Plants are half-erected with short to medium short shoots intensive colored. Leafs are larger, light green, half-open. This is a high yield cultivar.

5.4 Soil and weather analyses

Soil sampling on L1 shows neutral to medium alkali pH reaction (7.79 in H₂O and 6.59 in KCl). Level of humus at 2.55% was not satisfying. Relative low NH₄ of 0.98 mg per 100 g soil and NO₃ of 0.32 mg per 100 g soil have measured. Level of P₂O₅ of 35.9 mg per 100 g soil and K₂O of 24.5 mg per 100 g soil were satisfying. Anthropogenic soil on alluvial deposit with sandy loam texture was noticed.

Soil sampling on L2 shows neutral to medium alkali pH reaction (7.67 in H₂O and 6.43 in KCl). Level of humus at 2.55% was low. Relative low NH₄ of 0.98 mg per 100 g soil and NO₃ of 0.32 mg per 100 g soil have measured. Level of P₂O₅ of 17.5 mg per 100 g soil and K₂O of 20.0 mg per 100 g soil were satisfying. Soil texture on this location is classified as clay loam.

L3 shows medium acid pH soil reaction (6.56 in H₂O and 5.11 in KCl). Level of humus at 2.41% was low. Relative low NH₄ of 0.78 mg per 100 g soil and NO₃ of 1.24 mg per 100 g soil have measured. Very poor level of P₂O₅ of 0.40 mg per 100 g soil was detected while K₂O of 18.4 mg per 100 g soil were satisfying. Heavy mechanical soil composition on this location was noticed with poor water/air ratio and it was classified as loamy to loamy clay soil type.

Weather condition on three locations was measured from nearest local weather station. Colleted data on average monthly temperatures (°C), precipitation (L m⁻²) and air humidity (%) were shown for location Mostar (Figure 3), Malo Polje (Figure 4) and Stolac (Figure 5) in text.

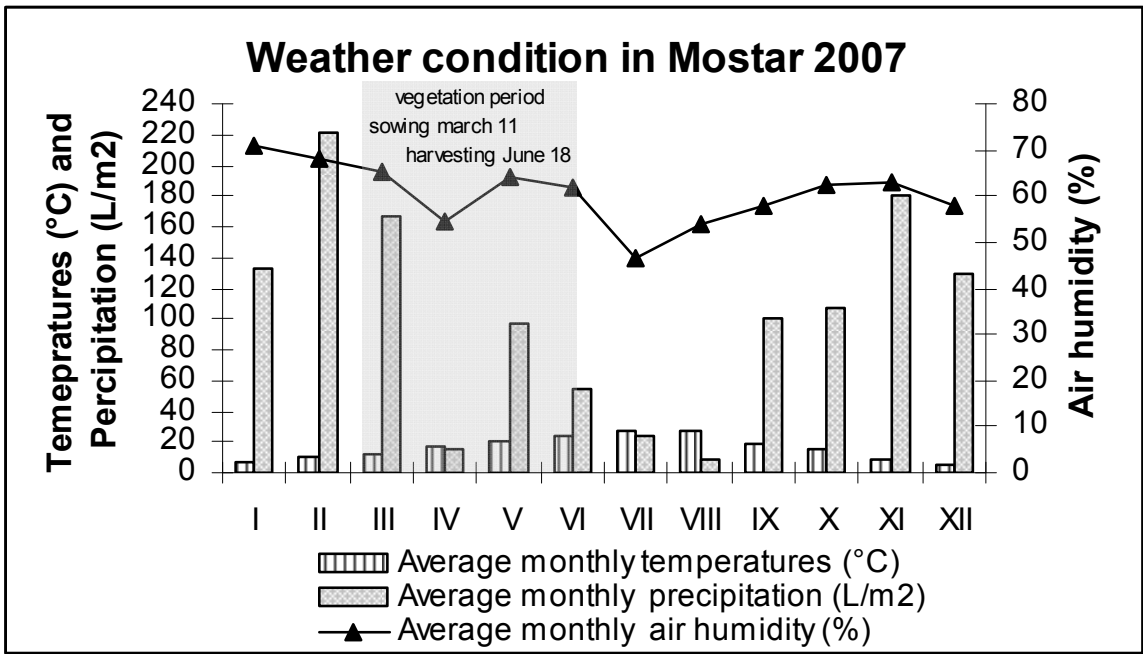


Fig. 3. Average monthly temperatures (°C), precipitation L m⁻² and air humidity (%) for Mostar with marked gray part for vegetation period form sowing date March 11 till harvesting June 18

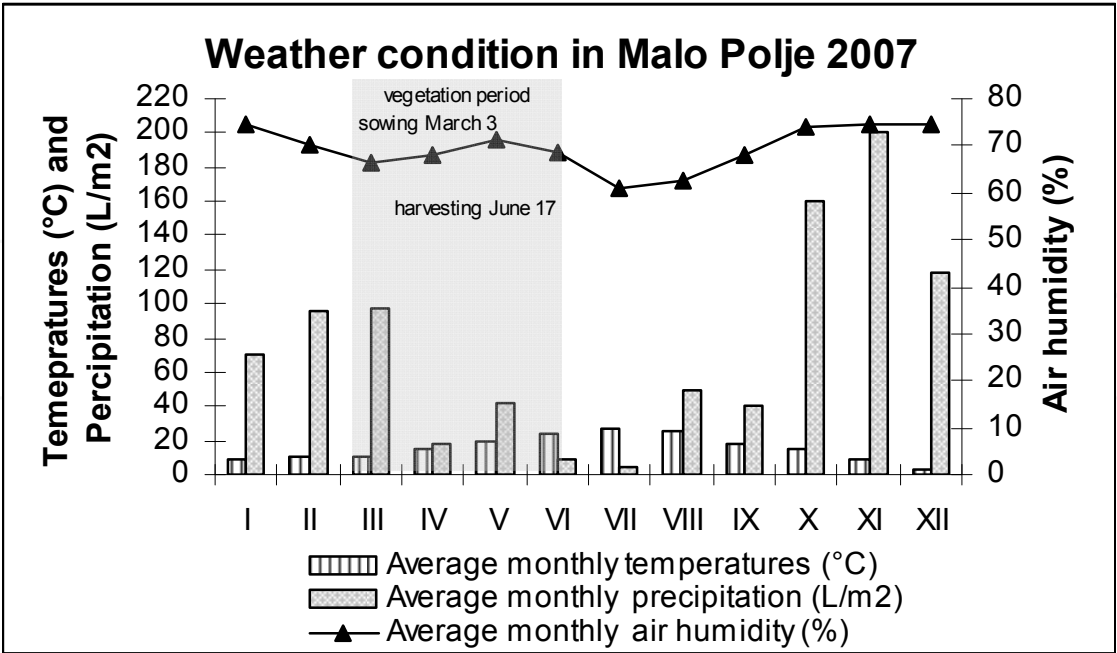


Fig. 4. Average monthly temperatures (°C), precipitation L m⁻² and air humidity (%) for Malo Polje with marked gray part for vegetation period form sowing date March 3 till harvesting June 17

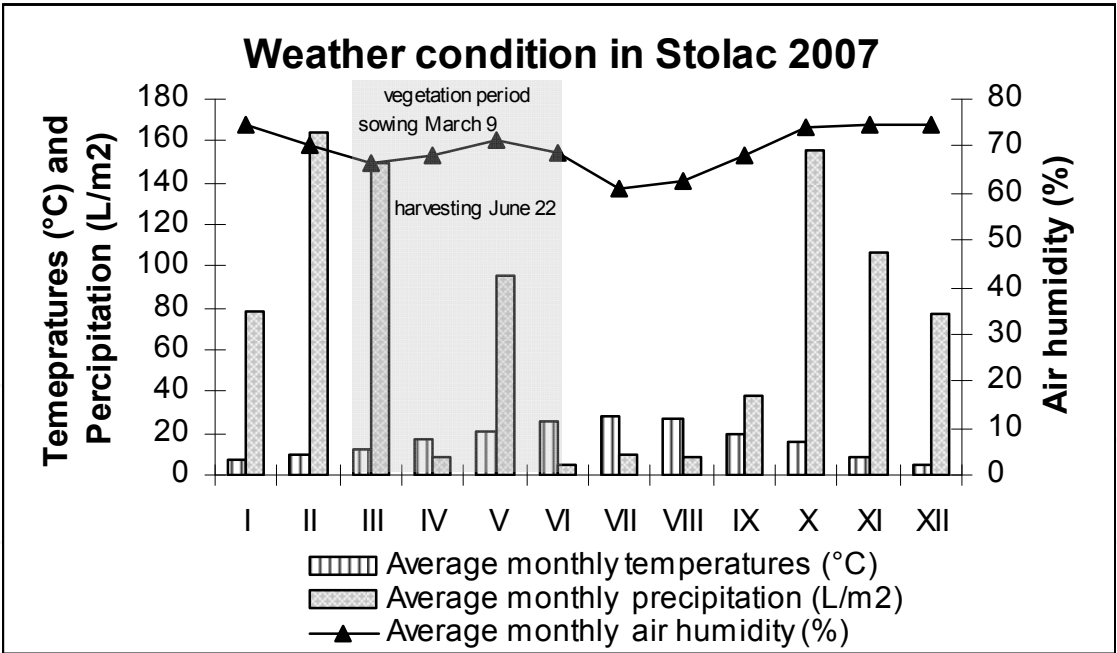


Fig. 5. Average monthly temperatures (°C), precipitation L m⁻² and air humidity (%) for Stolac with marked gray part for vegetation period form sowing date March 9 till harvesting June 22

For each location Figures were shown also with data about vegetation periods with information on sowing and harvesting date. For L1, L2 and L3 sowing dates were on March 11, 3 and 9 and harvesting were on June 18, 17 and 22. During season 2007 dry weather period has occurred in phase on tuber initiation. The phase of tuberization and tuber soaking were followed by increasing precipitations period with final phase of tuber

maturation amount of precipitation was decreased. Small increment in all three measured weather parameters were noticed in L3 for main growing stages while L1 and L3 shows relative similar situation. Comparison of obtained values with ten years average data shows slight increment in all three measured parameters.

5.5 Statistical analyses

Analyses of variance (ANOVA) were used for testing differences in SPAD values, concentration of NO₃-N mg kg⁻¹ and percentage of total nitrogen in potato leaves. Pearson’s correlation coefficient (*, **, p <0.05 and p <0.01) model was used for identifying correlations between: the total nitrogen (determinate on dry matter basis) and values collected with meters. Data were analyzed by using SPSS for Windows v 13.0 (SPSS, 2004). Our research objective was to determine how accurately the two meters estimated potato leaf N concentration. Chlorophyll (SPAD-502 meter, Minolta, Osaka,. Japan) and Cardy ion meter readings were compared with actual leaf N concentration (obtained using the Kjeldahl method) determined by laboratory plant analysis.

6. Results and discussion of plant analysis measurement on potato crop

Chlorophyll and Cardy ion readings have follow the nitrogen fertilization rate showing the good correlation relations to the total nitrogen measured in same samples (Table 1). Changes in values were expected regarding a variety and experiment location. Irrigation of the trial field was also provided according to the weather conditions.

	Days after sowing (DAS)				
Correlation coefficient (r) between values	65	75	85	95	Locations
N & NO ₃ -N	0.88**	0.71**	0.41*	0.24ns	Jasenica
	0.59**	0.59**	0.73**	0.14ns	Malo Polje
	0.62**	0.91**	0.85**	0.53**	Solac
N & SPAD	0.73**	0.59**	0.45*	0.43*	Jasenica
	0.37ns	0.67**	0.75*	0.32ns	Malo Polje
	0.31ns	0.75**	0.65*	0.73**	Solac
SPAD & NO ₃ -N	0.91**	0.75**	0.73**	0.46*	Jasenica
	0.61**	0.59**	0.64**	0.54**	Malo Polje
	0.35ns	0.72**	0.71**	0.68**	Stolac

Table 1. Correlations (r) between total N analysed on dry mater basis (Kjeldahl method) of potato leaf and NO₃-N values detected by Cardy-ion meter in petiole sap of potato; Correlations (r) between total N analysed on dry mater basis of potato leaf and SPAD values detected by chlorophyll meter; Correlation (r) between NO₃-N and SPAD values on three different locations during four sampling period (ns-non significant; * - significant at P=0.05; significant at the P=0.01)

Correlation relationships between SPAD and NO₃-N values obtain at all three location shows very significant coefficient for every sampling period. Other comparisons between N and NO₃-N as well and N and SPAD values haven't show positive coefficient during the all sampling period. Usually non significant coefficients were spotted at the beginning or at the end of the sampling period. The amount of chlorophyll a and b has been investigated in many studies and shown to correlate closely with leaf %N and SPAD meter value (Neukirchen et al., 2002). Vos & Bom (1993) have carried out the experiment with potato varieties Vebeca fertilized with 0, 110, 180 and 250 kg N ha⁻¹ in split application and they have compared a data of SPAD and NO₃-N values with standards. From the results they have confirmed a good correlation relation between SPAD values and chlorophyll with coefficient r=0.97. They have also confirmed good correlations between SPAD values and N while between SPAD and NO₃-N values coefficient was low. Gianquinto et al. (2004), in their investigation center in Scotland try to find a strong link between SPAD values and total leaf nitrogen. Very high correlation coefficient was established during the middle of the vegetation season while at the beginning and end of the season relationship was week. This survey has try to identify the nitrogen concentration using a rapid diagnostic tools for plant analysis as SPAD meter and nitrate level by Cardy ion meter in potato leaf during a different growth stages. Results of the ANOVA test for nitrogen concentration in potato leaf are shown in table 2.

Source of variability	Days after sowing (DAS)			
	65	75	85	95
	F-Test			
Location (L)	**	*	**	**
Cultivar (C)	**	**	**	*
Fertilization (F)	**	**	**	**
L × C	*	ns	ns	ns
L × F	ns	*	**	**
C × F	ns	ns	ns	ns
L × C × F	ns	ns	ns	ns
Fertilization treatment				
Control	3.53	3.19	2.86	2.38
100 kg N ha ⁻¹	4.18	3.54	3.09	2.52
200 kg N ha ⁻¹	4.63	4.04	3.44	2.87
LSD _{0.05}	0.20	0.14	0.10	0.15
Cultivars				
Adora	3.90	3.42	3.01	2.37
Liseta	4.22	3.70	3.24	2.66
Romano	4.21	3.65	3.14	2.74
LSD _{0.05}	0.13	0.16	0.05	0.27

Table 2. Result of ANOVA test for N concentration in potato leaf during the 4 growth stages. (ns - non significant; * - significant at P=0.05; significant at the P=0.01)

Beside significant impact on N leaf concentration of all three factor used in the experiment (location, cultivar and different fertilization rate) it is very interesting to note interaction of location and cultivar for 65 DAS as well as interaction of location and fertilization treatment for other three growing periods (75, 85 and 95 DAS). This means for 65 DAS that the values of the nitrogen at all three locations and for all three cultivars has varied. It was also conclude that the interaction for 75, 85 and 95 DAS shows significant different N concentration in potato leaf for all three location and the nitrogen fertilization treatments have achieved different values in each location. Results of the ANOVA test for SPAD values in potato leafs are also shown in the table 3.

As for nitrogen it is noted significant impacts of each factor on SPAD values. Interaction of location and cultivar was achieved at the beginning and end of the season while interaction of location and fertilization treatment was significant for each growth stage. The first interaction means that the each cultivar shows different SPAD values for each location. This means that the each location with them specific microclimate and soil conditions could affect the results. The second interaction indicates that different fertilization treatments at each location caused the differences in SPAD values. Generally the L3 has obtained highest SPAD values, followed by L1 while L2 was usually presented for each growing stage with smallest SPAD values.

Source of variability	Days after sowing (DAS)			
	65	75	85	95
	F-Test			
Location (L)	**	**	ns	**
Cultivar (C)	**	**	**	**
Fertilization (F)	**	**	**	**
L × C	**	ns	ns	*
L × F	**	*	**	**
C × F	ns	ns	ns	ns
L × C × F	ns	ns	ns	ns
Fertilization treatment				
Control	44.6	38.9	37.1	32.4
100 kg N ha ⁻¹	48.1	43.3	40.8	35.1
200 kg N ha ⁻¹	49.7	46.2	44.7	38.3
LSD _{0.05}	0.64	0.99	1.31	1.35
Cultivars				
Adora	48.8	41.8	40.5	35.0
Liseta	45.2	40.8	39.2	33.6
Romano	48.4	45.8	42.8	37.2
LSD _{0.05}	0.68	1.25	1.17	0.96

Table 3. Result of ANOVA test for SPAD values in potato leaf during the 4 growth stages at all three locations. (ns - non significant; * - significant at P=0.05; significant at the P=0.01)

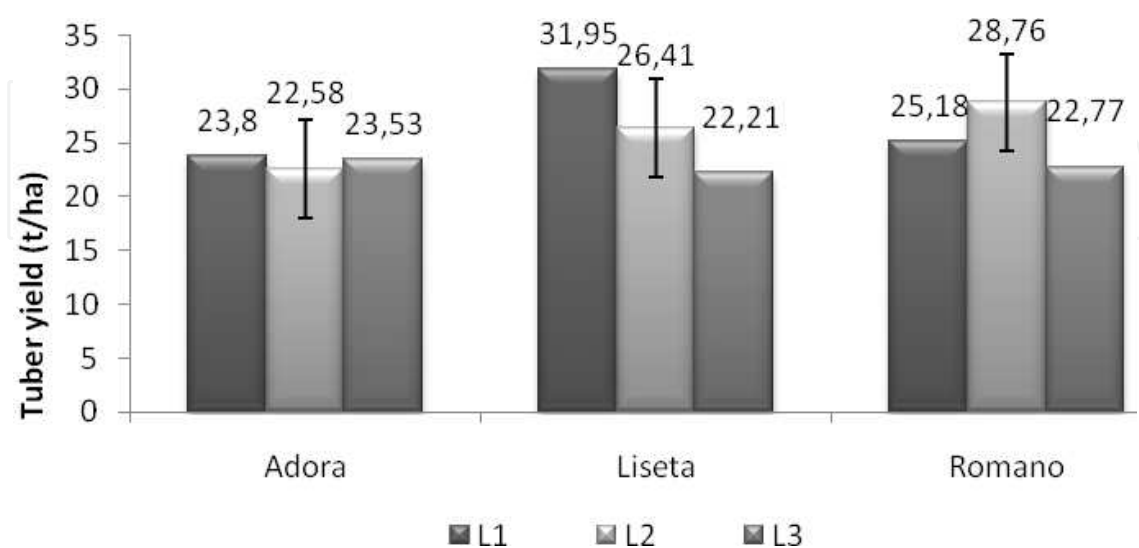
As we have obtained results of ANOVA test for N concentration and SPAD values, we have proceeded with the same test for NO₃-N values. Results of the ANOVA test for NO₃-N values measured by Cardy ion meter in potato leaf are also shown in the table 4. NO₃-N values are under the strong interaction between location and fertilization treatments while impact of location and cultivar interaction is noticed only on sampling at 85 DAS. Single factor impact on NO₃-N values for each growing stages was more expressed for location and for fertilization treatment while cultivar was significant only at the beginning of the season.

Source of variability	Days after sowing (DAS)			
	65	75	85	95
	F-Test			
Location (L)	**	*	**	**
Cultivar (C)	**	ns	ns	ns
Fertilization (F)	**	**	**	**
L × C	Ns	ns	*	ns
L × F	**	**	**	**
C × F	Ns	ns	ns	ns
L × C × F	Ns	ns	ns	ns
Fertilization treatment				
Control	543	244	107	154
100 kg N ha ⁻¹	1469	672	454	375
200 kg N ha ⁻¹	2069	1714	1186	985
LSD _{0.05}	160,4	201,4	144,6	229,9
Cultivars				
Adora	1481	860	614	683
Liseta	1418	852	576	352
Romano	1206	919	557	479
LSD _{0.05}	108,2	-	-	-

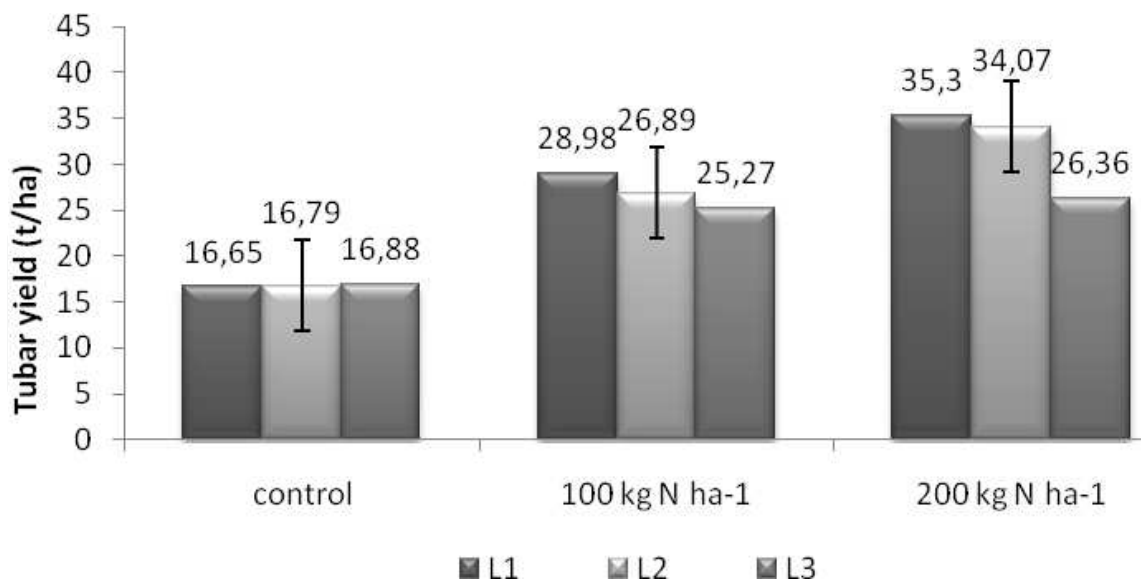
Table 4. Result of ANOVA test for NO₃-N level in petiole sap during the 4 growth stages at all Three locations. (ns - non significant; * - significant at P=0.05; significant at the P=0.01)

Average values shown in the table for N, SPAD and NO₃-N values were highest at L3. L1 has lower average values of N for 10%, SPAD for 9% and NO₃-N 54%. Comparing to L3 lower average values also has L2 for N 16%, SPAD 15% and NO₃-N 52%. If these measurements can serve to evaluate crop nutrient status it is logical that increscent or decrement of these values can influenced on crop yield. Even the average yield for each location statistically is not differing; the highest yield has achieved at L1, opposite from the expected highest results of measured values on location L3. These facts are explaining with environmental conditions on each location which were affected the nutrient availability and genetic potential of the crop. Cultivar Adora has average tuber yield of 23.30 t ha⁻¹, cultivar

Romano 25.57 t ha⁻¹ and Liseta has obtained the highest yield of 26.86 t ha⁻¹. Each location with their specific microclimate conditions can affect the yield formation of each cultivar which was confirmed interaction occurred between location and cultivar (graph 1). Same nutrient fertilization can result in different yield formation regarding to the location soil characteristic or weather conditions which was also confirmed interaction between location and fertilization treatments (graph 2). Also, genetic potential of each cultivar (vegetation period) can be affected by conditions on each location (e.g. temperature, light, precipitation, air moisture ect. (Gianquinto et al., 2006). Besides length of the potato growing period, early and late potato varieties differ in their dry matter accumulation and N assimilation rate (Kleinkopf et al., 1981). Reason for higher measured values at L3 can be explained by different soil reaction where this location has lower pH reaction comparing to other two. L3 have low acid to neutral soil pH reaction which was better for potatoes growing while other two locations have higher pH values. Average values of N, SPAD and NO₃-N were highest for first two sampling period and after that it was noticed slight decrement in values as the vegetation season passing. Reason for values diminishing with the time was that at beginning of season plants have intensive photosynthesis afterward they redistribute the nutrient from the canopy in the storage parts (tuber) which decrease protein content in leafs and increase in potato tuber (Millard & MacKerron, 1986). From our survey we can noticed that the cultivar with shortest vegetation period Adora has lowest N, SPAD and NO₃-N values because its earlier starts with redistribution of nutrient from canopy to the underground plants parts. Generally, earliest cultivar might required a different fertilization managements comparing to the latest potato varieties since tuberziation and N uptake occurs earlier in the season if they planted at the same time. In this case the amount of available N in soil can be very low when tuberization in later cultivar has occurred. A high application of N rate at the planting period should be avoided since the high amount of available nitrogen can delay potato tuber formation for 7 to 10 days (Kleinkopf et al., 1981) promoting the vegetation growth. This would be particularly important in areas with limited growing season.



Graph 1. The average tuber yield of cultivars Adora, Liseta, Romano on different locations L1, L2 and L3



Graph 2. Average tuber yield obtained by different fertilization rate on three location L1, L2 and L3

Gianquinto et al., (2004) have found that early cultivar shows higher SPAD values comparing to the later cultivar even the measurements were provided in the same period. From the data presented here, SPAD measurements were varied depending on cultivar. We have Romano cultivar with highest SPAD values, lower values have Adora and lowest were spotted in cultivar Liseta. Even Liseta cultivar showed highest N content the SPAD values were lowest while other two cultivars haven't shown significant variations in SPAD values. Reason for this was measurement procedure which implies recording a SPAD values in first full develop but not total matured leafs. Not all cultivar have same capacity for nutrient accumulation. If the measurement were not provided in the same vegetation period certain differences between total leaf N and SPAD values can be recorded even we have same samples. Debaeke et al. (2006) have conclude that the SPAD values highly depend on cultivar type, because not all cultivar have same thickens of the leaf as the thicker leaf shows higher chlorophyll units per leaf but not necessarily and higher N values. Beside cultivar type high impact on SPAD values have soil type, climate, water status in soil and plant. According to the Wheeler et al. (1989) decrement of the moisture content increase leaf surface and intensify leaf colour causing a higher SPAD values. In this survey applied fertilization treatments from 0 to 200 kg N ha⁻¹ showed average SPAD values for 65 DAS from 44,5 to 49,5; 75 DAS from 38,8 to 46,2; 85 DAS from 37,1 to 44,7; 95 DAS from 32,4 to 38,3 unit. Increasing nitrogen fertilization rate at all three locations have obtained increment of SPAD values and N values in crop also the values have diminished white time. These results were interpreted as good response of SPAD meter on N fertilization treatments and meter can serve as good tool in the evaluation of the plant nutrient status especially if we use a small control plot as reference strip. Nutrient concentrations decline ontogenetically during the growth period, even with sufficient N supply (Siman 1974; Sorensen, 2000). Therefore, the critical concentration has to be related to a carefully defined growth stage (Lorenz & Tyler, 1977). However, the way the growth stages are defined is often imprecise. Differences between SPAD and N values obtained in potato leaf samples could be explained by weather condition. In year when the experiment was set up during the vegetation period

(April, May and June) the temperatures were very high with low precipitation. In period of tuber initiation the temperatures have exceed a ten years average (13.3°C in April) for 4°C. According to the Wheeler et al., (1989) decreased moisture content could cause increment of leaf surface and intensify leaf colour. As we have mentioned the sensitivity of SPAD meter on environmental conditions the high temperatures with low precipitations could cause small transpiration rate of nutrient and water trough the plant resulting in the small intensity of photosynthesis. Stress condition for plant can develop increment of SPAD values at same level as plants that have received a fertilization treatments and water supply (Gianquinto et al., 2004).

The Cardy ion measurement can also provide a currently nutrient status in plants, detecting the nitrate-nitrogen content that was not yet incorporated in to organic compounds. In potatoes the Cardy ion measurements were widely accepted procedure to enable a quick assessment of the crop canopy nitrogen N status to derive N-fertiliser recommendations. Generally, Cardy ion measurements are more sensitive for detecting the N status of a crop compared to the chlorophyll analysis. This is because the nitrate concentration reacts more rapidly than the total N concentration to changes in the N supply (Huett and White, 1992). Cultivar with shorter vegetation period Adora and Liseta have shown the higher NO₃-N content only for measurement on 65 DAS while after Romano (longer vegetation period) variety have Obtained higher values as the varieties were not in the same vegetation phase. As the nitrate concentration varied regarding to the type of cultivar we have also found a good response of NO₃-N values on N fertilization treatment. Each treatment has shown differences in the NO₃-N values. Comparing to SPAD meter where values were smaller so the differences were mainly recorded between the fertilized treatment and control (no fertilization). The NO₃-N values for 65 DAS were from 543 to 2069 mg kg⁻¹ fresh weight; for 75 DAS from 244 to 1714 mg kg⁻¹; for 85 DAS 107 to 1186 and for last period of sampling 95 DAS we reached the values from 154 to 985 mg kg⁻¹ fresh weight. According to the Love et al., (1999) fertilization treatments from 0 to 250 kg N ha⁻¹ applied to Russet Burbank potato variety have obtained NO₃-N concentration from 22000 to 24000 mg kg⁻¹ dry weight in period of early tuberization and at the end of the vegetation season the values were from 2000 to 6000 mg kg dry weight. Differences between measurement in fresh and dry matter are large. For the Russet Burbank NO₃-N concentration in fresh weight have flouted from 1300 to 1600 mg kg⁻¹ in early maturation period while at the end of the vegetation the concentration have decreased on 550 to 700 mg kg⁻¹ fresh weight. In our experiment small variation were noticed for the NO₃-N values recorded in petiole sap during the four growth stages. On 85 DAS values shows lower NO₃-N concentration then on the last sampling period 95 DAS which was not in the accordance to the pervious statement of diminishing values as vegetation period ends. We explain this with strong influence of environmental condition where at the end of the season the higher precipitations have occurred. Under normal growing condition (with no excessive or any precipitation, extremely high or low temperatures and irradiation) nitrate nitrogen taken up by plants is readily converted into amino acids and proteins. Hence, the level of nitrate is not high enough to be toxic. During prolonged periods of moisture stress (drought) and high temperatures or low humidity, nitrate accumulates in plants (Malakouti, 2002). The severity of nitrate accumulation in plants is accentuated by heavy nitrogen fertilization prior to the onset of drought. Soil N mineralization, N fertilization rate, sampling date, and cultivar can influence the petiole N level (Vitosh & Silva, 1996). Insufficient water for plant growth can result in the

accumulation of $\text{NO}_3\text{-N}$ in petioles (Meyer & Marcum, 1998). Potatoes that have not received current season nitrogen fertilization generally have lower petiole N values earlier in the season and these values decline rapidly after tuber set (Gardner & Jones, 1975; Porter & Sisson, 1991; Wescott et al., 1991). It has been suggested since the rate of petiole N change during the season so rapid establishing of critical petiole N would be difficult unless the precise age of the plant is known and since the soil $\text{NO}_3\text{-N}$ status less fluctuates it might could be the better indicator of the N status of the crop (Doll et al., 1971; Rodrigues, 2004). More often sampling in closer intervals earlier in the season might have facilitated interpretation of petiole N trends.

According to the location influence on nitrate values the highest were recorded at L3 followed by L1 and the lowest were at L2. Fertilization treatments have significantly influenced on $\text{NO}_3\text{-N}$ values but the interaction of fertilization and location has shown that same fertilization treatments have achieved the highest values at L3. The lowest values of all measured parameters on L2 are defined by vary unfavorable microclimatic conditions where no participations have occurred followed by high temperatures during intensive potato growth. This variation in values between locations were rather connect to the weather then soil conditions where soil analyses have shown similar nutrient and other soil parameters at L1 and L2. So according to the soil parameters differences in values should not be occurred. Regarding to the soil nutrient availability it is important beside N application rate the residue cover, soil aggregation and structure, soil organic matter, crop rotation. These are the factor which can often result in lower nitrogen application rate, better use of applied nitrogen or less nitrate leaching.

A monitoring program is suggested due the variability in values from one site to the next and in order to measure the actual effects of soil N availability during growth (Wescott et al., 1991). Petioles have often been used for quick tests to estimate nitrate-N in sap (tissue nitrate-N). The quick tests have been developed for field use to avoid the time lag between sampling and result as well as the costs of laboratory analysis. Petiole plus midrib nitrate has been shown to reflect the N status, as for example in potato (Bélanger et al., 2003). Generally, nitrate concentrations tend to be higher in stems and petioles than in leaves so the nitrate level of vegetables that their petioles, stems or leaves are consume must be considered. Nitrate accumulation in older organs is high because activity of nitrate reductase in these parts is low (Malakouti, 2002). Nitrate and nitrite are detrimental components in plants after applying of nitrogen fertilizers in soil and oxidation by microorganism, the produced nitrate have high affinity to absorption by plants (Hogg et al., 1992; McKnight et al., 1997).

7. Conclusion

Data presented here shows response of the potato plant to the available nitrogen rate as an important factor for accurate fertilization practice. Obtained data confirm and extended previous studies which showed that chlorophyll measurement in combination with Cardy ion meter can be used in field conditions to detect differences in the response of a potatoes genotype to nitrogen supply. However, growers should understand that the chlorophyll meter is only able to reveal deficiency situations. Cardy ion meter readings are accurate enough to be used on a practical basis as a decision-making tool that can increase the efficiency of fertilizer use. If these two meters are calibrated correctly, it's possible to

conclude plant required N amount. Chlorophyll meter and Cardy ion meter are most effective when used in conjunction with other agronomic tools or agronomic knowledge. We also suggest that the N fertilization recommendations should be develop for each potato varieties. This practice for chlorophyll and Cardy ion measurement should be useful in management strategies which maximize use of previous crop residues, organic amendments and soil reserves as N sources. In such a system, at-planting N fertilizer applications would be reduced and supplemental N application could be applied when values of leaf chlorophyll or nitrate monitoring are beyond critical one for analysed crops taking care about environmental impact to recorded values. Analytical procedures for soil and plant analyses widely varied from location to location including and other factors (weather conditions, crop variety etc.); it is important to use test procedures calibrated for each geographical area or crops of interest. These nitrogen-monitoring techniques enable growers to apply nitrogen fertilizer at the right time, helping to ensure high yields without making unnecessary applications that could adversely affect the environment or possibly increase some pest damage. This also can save farmers money. The principal advantage to these techniques is the ability to get immediate results. Other advantages are that the equipment is portable and easily available through catalogs. There are several disadvantages to using the meter. First, the results, though close, are not as accurate as measurements from an analytical laboratory. Second, the instruments are sensitive to environmental conditions and can give inaccurate or inconsistent readings if exposed to heat, direct sunlight, water, sand and dirt. Therefore testing of sap should take place indoors under controlled environmental conditions and the reader should be kept clean and dry while chlorophyll reading should be immediately preformed. The environmental conditions also include those that directly affect the plant, which include excessive rain or irrigation at time of testing. Additional research is needed to determine the optimum amount of N that can be applied for certain potato variety.

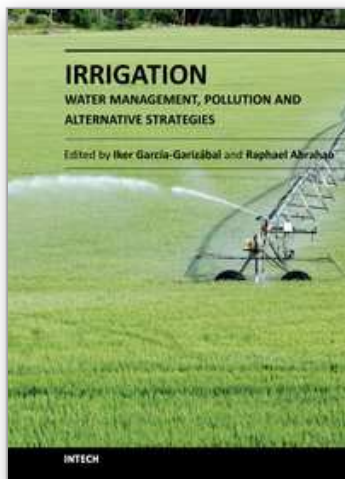
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Irrigation - Water Management, Pollution and Alternative Strategies

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Irrigated agriculture is the most significant user of fresh water in the world and, due to the large area occupied, is one of the major pollution sources for the water resources. This book comprises 12 chapters that cover different issues and problematics of irrigated agriculture: from water use in different irrigated systems to pollution generated by irrigated agriculture. Moreover, the book also includes chapters that deal with new possibilities of improving irrigation techniques through the reuse of drainage water and wastewater, helping to reduce freshwater extractions. A wide range of issues is herein presented, related to the evaluation of irrigated agriculture impacts and management practices to reduce these impacts on the environment.

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